a ligand of L-selectin; and

administering said agent to a mammal in need of such treatment so as to cause such inhibition to occur.

47. (New) A method for treating or inhibiting atherosclerosis in a mammal, comprising:

providing a first therapeutic agent for inhibiting interaction between P-selectin and a ligand of P-selectin;

providing a second therapeutic agent for inhibiting interaction between E-selectin and a ligand of E-selectin; and

administering said first agent and said second agent to a mammal in need of such treatment so as to cause such inhibition to occur.

REMARKS

The Office Action

Claims 1-13, 19, 20 and 23-39 are under examination. Claims 1-13, 19, 20 and 23-39 are rejected under 35 U.S.C. §103. Claims 1-12, 19, 20 and 26-39 are rejected under 35 U.S.C. §103. The specification has been amended. Claim 39 has been canceled. Claims 1, 5-9, 12, 13, 20, 24, 27-34 and 38 have been amended. New claims 40-47 have been added.

Specification

The Examiner has requested that the first paragraph of the specification be amended to indicate that parent case 08/253,663 is now abandoned.

Applicants have so amended the specification, and also added that the instant application is a continuation of 08/377,798.

New and Amended Claims

Applicants have canceled claim 39 and amended claim 1 such that the agent inhibits interaction between E-selectin and a ligand of E-selectin, as well as inhibiting interaction between P-selectin and a ligand of P-selectin. No new matter has been added. Independent claim 1 has simply been amended to incorporate the material in dependent claim 39. Support for this amendment is found in the specification at page 12, lines 21-26.

The original claims specifying only that the agent inhibit interactions between P-selectin and a P-selectin ligand have been amended to expedite prosecution of the present application, and their omission in the instant file wrapper continuation application should in no way be construed as an acquiescence to any of the Examiner's rejections for the parent application Serial No. 08/377,798. Applicants reserve the right to pursue claims containing agents which inhibit interactions between P-selectin and P-selectin ligands (without also requiring inhibition of E-selectin interactions) in separate continuation application(s).

Applicants have amended dependent claims 5-9, 12, 13, 24 and 27, in light of amended claim 1, to clarify that "ligand" in these dependent claims refers to a ligand of P-selectin. No new matter has been added.

Applicants have amended dependent claims 28-34 and 38, in light of amended claim 1, to include that the agent inhibits interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin. No new matter has been added.

Applicants have added new claim 40 to recite an inhibitory carbohydrate which is a heparin oligosaccharide. No new matter has been added. See original claim 20 which included heparin oligosaccharides in its Markush group. Amended claim 20 no longer includes heparin oligosaccharides.

Applicants have added new claims 41-43 to recite specific doses. Support for claim 41 is found in the specification at page 15, lines 22-24. Support for claim 42 is found in the specification at page 15, lines 16-18. Support for claim 43 is found in the specification at page 15, lines 19-21. No new matter has been added.

Applicants have added new claim 44 to recite a ligand of P-selectin which is a platelet. Support for this claim is found in the specification at page 5, lines 28-32, which states that P-selectin mediates adhesion of different types of cells to each other, e.g., heterotypic interactions of platelets or endothelial cells with blood cells.

Applicants have added new claim 45 to recite an agent which also inhibits interaction between L-selectin and a ligand of L-selectin. Support for this claim is found in the specification at page 12, lines 21-26. No new matter has been added.

Applicants have added new claim 46 which provides for an agent that inhibits interaction between P-selectin and a ligand of P-selectin, and between L-selectin and a ligand of L-selectin. Support for this claim is found in the specification at page 12, lines 21-26. No new matter has been added.

Applicants have added new claim 47 which provides a first agent for inhibiting interaction between P-selectin and a ligand of P-selectin, and a second agent for inhibiting interaction between E-selectin and a ligand of E-selectin. Support for this claim is found in the specification at page 13, lines 9-10, which recites that an agent (which inhibits interaction between P-selectin and a P-selectin ligand) can be administered in combination with other therapeutic agents. See also the specification at page 12, lines 22-24, which recites that E-selectin interactions can be the target for inhibition. No new matter has been added.

35 U.S.C. §103

In the final Office Action for the parent application, claims 1-13, 19, 20 and 23-28 were rejected under 35 U.S.C. §103 as being unpatentable over Kogan et al., Rao et al., or Seekamp et al., in view of Ross, and claims 1-12, 19, 20 and 26-39 were rejected under 35 U.S.C. §103 as being unpatentable over Rohrer et al. in view of DeAmbrosi and further in view of Ross. Applicants have overcome these rejections by amending claim 1 so as to recite an agent which inhibits interaction between P-

selectin and a P-selectin ligand and between E-selectin and an E-selectin ligand. This amendment has been made to expedite prosecution of the present application and should in no way be construed as an acquiescence to the Examiner's rejection. Applicants reserve the right to pursue claims containing an agent which inhibits interaction between P-selectin and a P-selectin ligand in separate continuation applications.

The Examiner further states regarding claim 39 from the parent application (which has been incorporated into amended claim 1 in this continuation application) that:

> [I]nhibition of the interaction of E-seletin with its ligand would reasonably have been expected to be a property of the inventive agent, because of the known similarities between molecules which bind E-selectin and those which bind P-selectin. If the property of inhibition of E-selectin binding were not inherent in every agent of independent claim 1 [claim 1 in the parent], it would have been obvious to select such agents for the purpose of enhanced effectiveness, because it was known in the art that both E- and P-selectin are involved with cardiovascular disease and inflammation. (Emphasis added).

First, it is not inherent that every agent which inhibits interaction between P-selectin and a P-selectin ligand also inhibits interaction between E-selectin and an E-selectin ligand. See attached §1.132 Declaration by Wagner, ¶4. The Examiner has not presented any evidence which was used as the basis for concluding that it is inherent that such a P-selectin inhibitor also is an E-selectin inhibitor. And, without such evidence, the Examiner has not met his burden. See, e.g., Ex parte Levy, 17 USPQ2d 1461, 1464 (Bd. Pat. App. and Int'f. 1990), which states:

> In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic *necessarily* flows from the teachings of the applied prior art. (Emphasis added).

See also Continental Can Co. USA, Inc. v. Monsanto Co., 948 F.2d 1264, 1269, 20 USPQ2d 1746, 1749-50 (Fed. Cir. 1991), in which the Federal Court concluded:

> To serve as an anticipation when the reference is silent about the asserted inherent characteristic, such gap in the reference may be filled with recourse to extrinsic evidence. Such evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. (Emphasis added).

The Examiner, however, has offered no evidence whatsoever, which would indicate that inhibition of E-selectin is inherent in an agent which inhibits P-selectin, or that it would be so recognized by persons of ordinary skill in the art.

Second, it was known in the art at the time that the parent application was filed, that in fact there are agents which inhibit P-selectin but not E-selectin, and agents which inhibit E-selectin but not P-selectin. See, e.g., attached Exhibit A: Cecconi et al., J. Biol. Chem. 269:15060-15066 (1994)(describes agent which inhibits P-selectin but not E-selectin), and Exhibit B: Lenter et al., J. Cell Biol. 125:471-481 (1994)(describes ligands which bind to P-selectin but not to E-selectin and other ligands which bind to E-selectin but not to P-selectin). See §1.132 Declaration by Wagner, ¶4.

Third, it was not obvious at the time that the parent application was filed to select agents which inhibit both P-selectin and E-selectin for enhanced effectiveness in treating atherosclerosis. Contrary to the Examiner's assertion, it was not known in the art at that time that both E- and P-selectin are involved in cardiovascular disease and chronic inflammation. See §1.132 Declaration by Wagner, ¶5.

As is known by persons skilled in the art, an important distinction exists between "acute inflammation" and "chronic inflammation." See, e.g., attached Exhibit C: Robbins, in "Pathologic Basis of Disease," 5th Ed., R.S. Cotran, M.D., V. Kumar, M.D. and S.L. Robbins, M.D., W.B. Saunders Co., Philadelphia, PA, pp. 51-76 (1994). "Acute inflammation" is of relatively short duration and is involved in processes such as wound repair, infection and reperfusion injury. It involves mainly recruitment of neutrophils. "Chronic inflammation," on the other hand, is of longer duration and is associated predominantly with the recruitment of monocytes and T-cells. As is known to one skilled in the art, atherosclerosis is a special example of chronic inflammation. This specificity of recruitment, combined with smooth muscle cell proliferation and dependence on cholesterol ingestion by the monocytes/macrophages, makes atherosclerosis a unique process. At the time that the parent application was filed, there were no known adhesion receptors that were specific for recruitment of monocytes and T-cells. See §1.132 Declaration by Wagner, ¶6.

As was known to one skilled in the art at the time that the parent application was filed, P-selectin was a receptor that mediated rolling of many types of white blood cells, was rapidly expressed on activated cells, and was stored in preformed granules that could be rapidly released from these cells. Moreover, as was known by one skilled in the art at the time, P-selectin was involved in early recruitment of neutrophils in experimentally-induced inflammation. See attached Exhibit D: Mayadas et al., Cell 74:541-554 (1993)(recruitment of neutrophils was delayed in P-selectin-deficient mice for two hours and then occurred at a rate identical to wild-type mice). Similarly, delay in the recruitment of neutrophils in wound healing has been reported to occur only in the first two hours in P-selectin-deficient mice after injury. See attached Exhibit E: Subramaniam et al., Am. J. Pathology 150:1701-1709 (1997). And, recruitment of macrophages three to seven days post wounding has been reported to be normal in P-selectin-deficient mice, with wound healing occurring at the same rate as in wild-type mice. See attached Exhibit E: Subramaniam et al., Am. J. Pathology 150:1701-1709 (1997). To a person skilled in the art, these results indicate that P-selectin plays a role in acute inflammation and injury, but not in chronic processes such as atherosclerosis. See §1.132 Declaration by Wagner, ¶7.

The first published indications that P-selectin plays a role in long-term chronic inflammation came in 1995, after the filing date of the parent application. See attached Exhibit J: Johnson

et al., Blood 86:1106-1114 (1995), which showed reduced macrophage recruitment 48 hours after induction of experimental inflammation. See also attached Exhibit F: Subramaniam et al., J. Exp. Med. 181:2277-2282 (1995). This paper reported that recruitment of inflammatory cells, including $CD4^+$ T cells, in a contact hypersensitivity response, was reduced in P-selectin-deficient mice. This result was a big surprise to those skilled in the art. See §1.132 Declaration by Wagner, ¶8.

And, it was not until 1997, after the filing date of the parent application, that the first published report appeared demonstrating a role for any adhesion receptor molecule, and specifically for P-selectin, in atherosclerosis. See attached Exhibit G: Johnson et al., J. Clin. Invest. 99:1037-1043 (1997). See §1.132 Declaration by Wagner, ¶9.

In sum, a role for P-selectin in chronic inflammation such as atherosclerosis was contrary to the state of knowledge of those skilled in the art at the time that the parent application was filed, and was certainly not "obvious" to those skilled in the art. See §1.132 Declaration by Wagner, ¶10.

Moreover, a role for E-selectin in chronic inflammation such as atherosclerosis was not experimentally supported at the time that the parent application was filed. Indeed, even as of the instant date, no defects in any inflammatory or wound healing models have been reported for E-selectin-deficient mice unless antibodies inhibitory of P-selectin are also used. See attached Exhibit K: Labow et al., Immunity 1:700-720 (1994)(published

after the filing date of the parent application). See §1.132 Declaration by Wagner, ¶11. It was not until 1996, after the filing of the parent application, that it was reported by the inventors and others that major defects existed in P- and E-selectin double deficient mice. See attached Exhibit H: Frenette et al., Cell 84:563-574 (1996), and attached Exhibit I: Bullard et al., J. Exp. Med. 183:2329-2336 (1996). These papers showed conclusively for the first time that the two endothelial selectins, P and E together, are crucial for leukocyte recruitment to sites of inflammation. Prior to these papers, these selectins were known to be involved in leukocyte rolling (with minor or no consequences on leukocyte recruitment)(see attached Exhibit D: Mayadas et al., Cell 74:541-554 (1993)), and it was believed by persons skilled in the art that it was the adhesion molecules responsible for leukocyte firm adhesion to endothelium (belonging to the immunoglobulin and integrin family of receptors), that were crucial for the final transmigration of leukocytes to sites of inflammation in the tissues. See §1.132 Declaration by Wagner, ¶12.

Thus, a role for E-selectin in atherosclerosis had not been shown at the time that the parent application was filed. See §1.132 Declaration by Wagner, ¶13.

Attached hereto as Exhibit K are five figures illustrating the results of experiments performed by the inventors which support the surprising and unexpected results obtained from mice

being deficient in both P-selectin and E-selectin, as opposed to being deficient just for P-selectin, in inhibiting atherosclerotic lesions on arterial walls. Experimental protocols were performed as described in Exhibit G: Johnson et al., J. Clin. Invest. 99:1037-1043 (1997). Fig. 1 illustrates that the size of aortic sinus lesions in LDL-receptor (LDLR)-deficient mice on an atherogenic (high cholesterol and fat) diet is significantly smaller in P- and E-selectin double deficient mice than in wild-type or just P-selectin-deficient mice. Fig. 2 consists of photographs of entire aortae of LDLR-deficient mice on an atherogenic diet, and illustrates that the percentage area of the aortae that have atherosclerotic lesions is significantly smaller in P- and E-selectin double deficient mice than in wild-type mice. Fig. 3 illustrates that there are significantly smaller aortic sinus lesions in LDLR-deficient mice on an atherogenic diet in P- and E-selectin double deficient mice than in wild-type or just P-selectin-deficient mice. Fig. 4 illustrates that the size of atherosclerotic lesions in the aortic sinus of LDLR-deficient mice, as a function of the length of time on an atherogenic diet, is significantly smaller for up to at least 37 weeks, in P- and E-selectin double deficient mice than in wild- type or just P-selectin-deficient mice. Fig. 5 illustrates that the percentage of mice with calcification in the aortic sinus of LDLR-deficient mice on an atherogenic diet, is significantly less in P- and E-selectin double deficient mice than in wild type mice. See §1.132 Declaration by Wagner, ¶14.

In sum, no combination of the cited prior art teaches or suggests a method for treating or inhibiting atherosclerosis by providing an agent for inhibiting interaction between P-selectin and a ligand of P-selectin and between E-selectin and a ligand of E-selectin. Nor does any combination of the cited prior art suggest the advantages that are present in applicants' invention. See §1.132 Declaration by Wagner, ¶15.

Summary

In view of the above, it is respectfully submitted that the claims are in condition for allowance and such action is requested.

If the Examiner will not allow this application upon receipt and consideration of this amendment, it is respectfully requested that the Examiner call applicants' undersigned counsel prior to action in order to discuss the issues and advance the prosecution of the application.

Respectfully submitted,

By: Helen Greer

Helen Greer, Reg. No. 36,816
NUTTER, McCLENNEN & FISH, LLP
One International Place
Boston, MA 02110-2699
Tel (617) 439-2781

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